

Clinical Tests in Aquatic Toxicology: State of the Art

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Hazard assessment of chemicals to aquatic organisms involves the use of many toxicity tests. Acute toxicity tests, embryo-larval toxicity tests, and chronic toxicity tests that measure survival, growth, and reproductive effects now provide the most relative utility for evaluation of potential chemical hazards to aquatic life. Physiological, biochemical, and histological measurements have a low relative utility as diagnostic tests in aquatic toxicology because it is not yet possible to relate changes in these sublethal responses to adverse environmental impacts. The problem of interpreting the toxicological significance of chemical-induced changes in biochemical and physiological mechanisms is twofold: (1) the understanding of physiological and biochemical regulatory mechanisms in fish is limited; and (2) parallel changes in these mechanisms are difficult to correlate with toxicant exposure and impaired ability of fish to survive. To overcome this problem, more physiological and biochemical research must be conducted in conjunction with toxicity studies that measure important whole-animal responses. Toxicant-induced biochemical and physiological responses must be correlated unequivocally with responses related to reproduction, growth and development, survival, or fish health if pertinent diagnostic tests are to be developed for use in aquatic toxicology. The use of diagnostic tests in hazard assessment procedures can decrease the time required for safety evaluation of chemicals, define no-effect exposure concentrations more adequately, and provide a better understanding of the mode of action of chemicals. Considerations for improving the status of the "state of the art" of diagnostic or clinical tests in aquatic toxicology are discussed.

The pollution of the aquatic environment by agriculture and industrial chemicals, oil spills, mine effluents, and many other chemical contaminants has been recognized for many years. Approaches used to evaluate the health and well-being of fish subjected to environmental insults have in the past focused primarily on short-term studies utilizing whole-animal responses, such as gross abnormalities, behavioral changes, altered length and weight, and mortality. In mammalian and veterinary toxicology, in contrast, clinical techniques are used in conjunction with whole-animal responses to evaluate the health of animals exposed to toxic chemicals. These clinical techniques include blood chemistry profiles, blood cell analyses, and histopathological examinations of various organs and tissues. The use of these clinical diagnoses has been

established over the years by extensive research correlating physiological and biochemical responses with whole-animal responses. These correlations allow the mammalian toxicologist to interpret the biological significance of physiological responses induced by toxic chemicals.

Similar diagnostic tests are not available to fish toxicologists because biochemical and physiological research has been less extensive in aquatic toxicology, which is a relatively new field of science. The "state of the art" of physiological, biochemical, and histological tests in aquatic toxicology was recently summarized by participants in a workshop on aquatic toxicology held at Pellston, Michigan (1). The participants rated the relative utility of eleven toxicity tests using the criteria of ecological significance of effects, scientific and legal defensibility, availability of acceptable methods, utility of test results in predicting effects in aquatic environments, the general applicability to all classes of chemicals, and the simplicity and cost of the test. In terms of present utility for use in assessing the hazard to aquatic environments, acute lethality tests were

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rated highest, followed by embryo-larval tests, chronic toxicity tests measuring reproductive effects, and residue accumulation studies. Histological tests ranked ninth and physiological and biochemical tests tenth in overall and present relative utility because of the inability to relate the results of these tests to adverse environmental impacts. We summarize problems associated with developing diagnostic tests for fish, and discuss our philosophy on their development and application in aquatic toxicology.

Problems Associated with Developing Diagnostic Tests in Aquatic Toxicology

Development of diagnostic tests for use in aquatic toxicology, as well as application of mammalian clinical tests to fish, requires consideration of three important factors: (1) an understanding of the functions and composition of organs primarily affected by toxic chemicals, (2) knowledge of analytical procedures to measure appropriate biochemical constituents, cellular morphology, and physiological responses, and (3) ability to interpret the significance of chemical-induced biophysiological changes and relate them to fish health and survival. The first two factors have been addressed quite extensively in fishery research. A considerable number of biochemical, physiological, and histopathological investigations have been conducted on both freshwater and marine fish. Hunn (2) showed that many techniques for constituents have been developed for determining organic and inorganic constituents of fish blood, and values for the constituents reported. Similarly, histopathological techniques have been used on various tissues and organs to describe lesions caused by diseases, chemical and physical agents, and nutritional factors (3). However, the third factor has been very difficult to address in fishery research, and more specifically, in aquatic toxicology. Although clinical techniques for measuring physiological and biochemical changes in mammalian toxicology have been successfully applied to various fluids and tissues in fishes, chemically induced alterations have been of only limited use because changes usually have not been related to an impaired ability of fish to adapt or survive in natural habitats. The problem of interpreting the toxicological significance of chemically induced changes in biochemical and physiological mechanisms in fish is twofold: our understanding of physiological and biochemical regulatory mechanisms in fish is limited; and, parallel changes in these factors have not been correlated with toxicant exposure and

impaired ability of fish to survive. This lack of understanding encourages the extrapolation of mammalian clinical interpretations to responses observed in fish. However, the extrapolation of mammalian clinical response to poikilothermic animals is largely unproven and can lead to inaccurate interpretations of the status of fish health. Physiological and biochemical mechanisms in poikilothermic animals allow them to adapt to various environmental factors. These normal adaptive responses to environmental stresses such as toxic chemicals, diseases, water quality, and temperature fluctuations are reflected by changes in blood and tissue constituents, but deviations from physiologic "norms" that fish can tolerate are not well understood (4). Since variations in fluid and tissue constituents exist within a species of fish, as well as among species and populations, it is difficult to establish "normal" or baseline values which are needed to develop and apply diagnostic tests. Wedemeyer and Yasutake (5), who discussed this difficulty in their review on the use of clinical methods to assess the influence of environmental stress on fish health, reported the expected concentration ranges and the possible significance of changes in blood and tissue constituents of salmonids. However, many factors unrelated to environmental stresses and diseases have been shown to alter concentrations of blood constituents. Barnhart (6) showed that diet, strain, and age affected hematological characteristics of rainbow trout (*Salmo gairdneri*). Also, the stress of handling, anesthesia, and various methods of capture significantly altered blood characteristics in salmonids (7-9). These studies illustrated that normal or baseline concentrations of hematological and tissue constituents in fish are difficult to determine and are a major obstacle facing the aquatic toxicologist attempting to develop diagnostic tests.

An equally difficult problem to overcome is unequivocal correlation of toxicant-induced physiological and biochemical responses with an important whole-animal response critical to species or population survival (e.g., reproduction, growth and development, or adaptability). Studies at the Columbia National Fisheries Research Laboratory have illustrated this problem very well. In rainbow trout exposed to sublethal concentrations of dietary endrin for 165 days, significant changes occurred in serum sodium, chloride, osmolality, total protein, cholesterol, cortisol, lactate, glucose, and liver glycogen (10). The major conclusions were that glycogenolysis and the cortisol stress-response mechanism were inhibited, which implied decreased survival because the ability of the trout to respond to environmental stresses had been reduced. Although the implication was correct, similar biochemical manifestations of

the stress-response mechanisms can be induced by other nonspecific stresses such as fish handling and temperature shock (11, 12). To interpret adequately the significance of the endrin-induced changes, investigators must have a better understanding of the extremes that trout can tolerate biochemically in stress-response mechanisms. This understanding would better enable them to predict, by diagnostic tests, the eventual effects on fish of contaminants such as endrin.

In another study where rainbow trout were exposed to sublethal dietary dieldrin concentrations for 300 days (13), liver phenylalanine hydroxylase activity was decreased and blood phenylalanine concentrations and urine concentrations of phenylpyruvic acid were increased. These responses were similar to the biochemical manifestations of phenylketonuria (PKU), an inherited metabolic disorder in humans. The disease is characterized by altered behavior and learning ability. However, since behavioral studies were not conducted with the trout, the significance of the biochemical manifestations of PKU in fish could not be assessed.

Christensen et al. (14) reported that hematological characteristics were altered in brook trout (*Salvelinus fontinalis*) exposed to heavy metals: lead increased hemoglobin and glutamic-oxaloacetic transaminase activity; cadmium increased plasma chloride and lactate dehydrogenase activity and decreased plasma glucose; methylmercury increased plasma sodium and chloride and hemoglobin. These biochemical changes were used as an early sign of toxicity to derive no-effect exposure concentrations, although the changes could not be directly related to any adverse effect on the health of the fish. However, the authors discussed the need to better understand cause-effect relationships at the molecular, tissue, and organ levels if biochemical changes are to be used as diagnostic tests for predicting threshold toxicity concentrations.

The studies mentioned above are by no means a comprehensive review of the biochemical and physiological effects of toxic chemicals on fish, but show that to interpret contaminant-induced biochemical and physiological changes, it is essential that these changes be related to impairments affecting fish health or survival. Biochemical indicators and effects monitoring for regulatory purposes and hazard assessment in aquatic toxicology cannot rely on unsupported assumptions or extrapolations from mammalian toxicology and human medicine. These extrapolations can lead to broad, erroneous conclusions in interpreting contaminant effects in poikilothermic animals whose biochemical adaptive capacities are not completely understood.

Considerations for Developing Diagnostic Tests in Fish Toxicology

The preceding discussion has shown that the state of the art of clinical analyses or diagnostic tests in aquatic toxicology is not well advanced. As we have indicated, the analytical techniques and instrumentation are well developed for performing clinical analyses, and considerable research on physiological and biochemical responses induced by chemical toxicants has been conducted, but useful biological or diagnostic indicators have not been developed. In our opinion, the main reason for this lack of progress has been the lack of a comprehensive, integrated approach in toxicological studies with fish. To overcome this problem, researchers must conduct biochemical, physiological, and histopathological investigations in conjunction with toxicity studies that measure important whole-animal responses, and the responses must be unequivocally related to whole-animal responses. Establishing this relationship will help insure development of pertinent diagnostic indicators of fish health. The choice of whole-animal responses to evaluate in toxicity studies with fish depends on the purpose of the toxicology program, but in our program, as well as in many other aquatic toxicology programs, emphasis is given to toxicant effects on survival, growth and development, reproduction, and adaptability.

It recently has been concluded (1) that the most meaningful information for hazard assessments of a wide variety of chemicals in aquatic environments is laboratory toxicity tests such as those for acute lethality, partial chronic toxicity (embryo/larval), chronic toxicity (including reproduction), and residue accumulation. Physiological and biochemical tests are generally not conducted for two reasons: it is felt that they are mainly useful in evaluating the mode of action of chemicals (15); or not enough basic information is available about fish physiology and biochemistry to ascertain the ultimate effects, since alterations in these processes do not necessarily indicate a disadvantage to the survival and success of the organisms. To assess the influence of contaminants on the aquatic environment adequately and to overcome the avoidance of biochemical and physiological testing, investigators should develop techniques that can serve as biological indicators in the field as well as predictors in the laboratory to estimate the "health" of a particular aquatic resource. However, biochemical and physiological changes must be viewed in light of the degree and duration of change to determine whether the or-

ganism can adapt or whether the changes lead to irreversible homeostatic disturbances and finally to the death or debilitation of the organism.

For the past several years, we have studied the use of biochemical and physiological tests to predict and monitor effects of contaminants on growth and development in fish. The emphasis of these studies was on the unequivocal correlation of a biochemical response to the whole-animal response of growth. Growth of fish is usually evaluated by measuring weight or length. However, growth is the culmination of many biochemical phenomena occurring in a somewhat regulated pattern, and biochemical changes due to contaminant intoxication should occur before reductions in growth are observed. Appropriate tests might therefore decrease the time required for chronic toxicity determinations. Initially, we selected vertebral phosphorus, calcium, and collagen content and the hydroxyproline and proline concentrations in collagen as potential indicators of growth and development in fish. Since fish growth is indeterminate and vertebrae continue to elongate and enlarge throughout life, we hypothesized that vertebral development (e.g., changes in organic and inorganic constituents) could be correlated with growth of the fish. The aforementioned physiological and biochemical characteristics of fish vertebrae were measured and evaluated in partial chronic and chronic toxicity studies of toxaphene, an organochlorine insecticide (16-18); dimethylamine salt of 2, 4-D, a phenoxyacetic acid herbicide (19); di-2-ethylhexyl phthalate, a phthalic acid ester plasticizer (19); Aroclor 1254, a polychlorinated biphenyl dielectric fluid (20); and Pydraul, an organophosphate hydraulic fluid (unpublished data, Columbia National Fisheries Research Laboratory). In these studies, we found that vertebral collagen and hydroxyproline concentrations were sensitive indicators of growth and bone development. In fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*) exposed to toxaphene, alterations in vertebral collagen metabolism were observed prior to reduction in growth or altered vertebral structure. Significant correlations were observed between fish weight and vertebral collagen and hydroxyproline concentrations. Similarly, in fish exposed to the other chemicals, changes in collagen metabolism were observed before reduction in growth, and at lower exposure concentrations. The correlations between growth and vertebral composition and between vertebral composition and backbone structure make vertebral collagen measurements very promising as diagnostic tests for contaminant-induced effects on growth and development in fish.

These chronic toxicity studies have illustrated an

important point concerning chemical residues in fish. Determination of residue accumulation in assessing environmental persistency of contaminants has real application, but direct correlations of whole-body residues with biological responses may be questionable. We found an overall decrease in collagen metabolism in relation to increased toxicant residues; however, the correlation was very poor. For example, in channel catfish exposed to toxaphene, only an additional 11% decrease in collagen was found after increasing both the exposure concentration and the whole-body residue 15-fold over that of the no-effect concentration. Once an exposure concentration that induced an effect or a whole-body residue was reached, little additional response was noted. Therefore, whole-body residue concentration does not necessarily reflect the status of fish health. However, determining the residue dynamics of chemicals among tissues such as liver, depot fat, muscle, and brain may be more fruitful than measuring whole-body concentrations.

We believe that one of the major principles overlying efforts toward developing indicators is the need to plan and execute the biochemical and physiological research within the framework of what exists in the "real world." Unless the status of the environment we are attempting to improve and preserve is kept in proper perspective, much of our efforts will be of little or no avail. Consequently, the continued reassessment of aquatic toxicology in relation to the continued change in environmental needs is mandatory if the obvious objectives are to be reached. Yet, we must not forget the great need for basic knowledge when research programs are designed, especially with aquatic organisms, for this information is ultimately the foundation for applied research.

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